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13. ABSTRACT (maximum of 200 words) Early experiments included sharp-intracellular-electrode analyses of amino-acid-mediated synaptic transmission and intrinsic membrane properties, designed in part to reveal the degree to which SCN neurons are homogenous or heterogenous. This work showed that glutamate and GABA play critical roles in synaptic transmission in the SCN, and that SCN neurons are not homogenous in terms of their electrophysiological properties, although they could not be grouped into distinct neuron classes. Multiple-unit extracellular recordings showed synchronous bursts of action potentials in the SCN in low [Ca ²⁺] solutions containing amino-acid-receptor antagonists (demonstrated to block chemical synapses), thus suggesting that SCN neurons are capable of communicating through nonsynaptic mechanisms. Whole-cell patch-clamp data showed that SCN neurons have a novel delayed outward-rectifier K ⁺ current and a transient K ⁺ current (i.e., A-current), both of which are present in all SCN neurons. More recently, we have studied local synaptic circuits and GABA-mediated inhibition in the SCN. Using glutamate microapplication to selectively stimulate only SCN neurons, we have provided physiological evidence that SCN neurons are interconnected by inhibitory circuits.			
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**Final Technical Report
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Cellular Neurophysiology of the Rat Suprachiasmatic Nucleus
Electrical Properties, Neurotransmission and Mechanisms of Synchronization
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1. RESEARCH OBJECTIVES

The focus of our research is to test hypotheses about the electrophysiological properties and local synaptic interactions of neurons in the suprachiasmatic nucleus (SCN). Our long-term goals are to understand the mechanisms responsible for modulating the electrical activity and the circadian rhythm of SCN neurons, and to find pharmacological methods to modulate these mechanisms. Our research approach to study these mechanisms has been at the single-cell and network levels. Using hypothalamic slices, we aimed to determine the electrical properties of SCN neurons, whether single SCN neurons have circadian changes in membrane properties, and how SCN neurons communicate with each other. Our primary specific aim near the end of the grant was to identify the synaptic mechanisms of neuronal communication within the SCN that contribute to the coordinated circadian rhythm.

2. STATUS OF EFFORT

Our early experiments included sharp-intracellular-electrode analyses of amino-acid-mediated synaptic transmission and intrinsic membrane properties, designed in part to reveal the degree to which SCN neurons are homogenous or heterogeneous. This work showed that glutamate and GABA play critical roles in synaptic transmission in the SCN, and that SCN neurons are not homogenous in terms of their electrophysiological properties, although they could not be grouped into distinct neuron classes. These experiments were conducted with Yang Kim at UCLA, and are included here because the final stages of the manuscript were accomplished with this grant.

Multiple-unit extracellular recordings showed synchronous bursts of action potentials in the SCN in low $[Ca^{2+}]$ solutions containing amino-acid-receptor antagonists (demonstrated to block chemical synapses), thus suggesting that SCN neurons are capable of communicating through nonsynaptic mechanisms. Whole-cell patch-clamp data showed that SCN neurons have a novel delayed outward-rectifier K^+ current and a transient K^+ current (i.e., A-current), both of which are present in all SCN neurons. These studies were conducted with Yona Bouskila, and were a major focus of this grant.

More recently, we have studied local synaptic circuits and GABA-mediated inhibition in the SCN. Using glutamate microapplication to selectively stimulate only SCN neurons, we have provided physiological evidence that SCN neurons are interconnected by inhibitory circuits. We intend to determine the spatial organization of these local circuits, and to test the hypothesis that these circuits synchronize and augment the circadian rhythm of neuronal activity in the SCN. We have also studied the effects of Zn^{2+} and benzodiazepines on $GABA_A$ -receptor-mediated synaptic currents: we found that Zn^{2+} is a moderately effective blocker of GABA-mediated responses in the SCN, and that benzodiazepines prolong GABA-mediated IPSCs and augment responses to exogenous GABA. These experiments were primarily conducted with Joe Strecker, and they have been extended with our ongoing AFOSR grant.

Finally, we have reviewed a series of experiments on the paraventricular nucleus, supraoptic nucleus, and the preoptic area; these studies were only partially supported by the AFOSR and are included because they complement the research on the SCN.

3. ACCOMPLISHMENTS/NEW FINDINGS

A. Suprachiasmatic nucleus (SCN)

(i) Membrane properties of SCN neurons examined with intracellular recordings

Sharp-electrode intracellular recordings had been obtained at UCLA to determine whether SCN neurons had homogenous or heterogenous electrophysiological properties. Most of our efforts were aimed at recording from neurons that received retinal input. A quantitative analysis was undertaken of resting potential, input resistance, action potentials, and hyperpolarizing afterpotentials; these properties were similar across SCN neurons. Other properties, however, differed between neurons. We found that some neurons showed time- and voltage-dependent inward rectification, and some of them had low-threshold Ca^{2+} spikes. We also found that the firing pattern (i.e., regular versus irregular) was directly related to the firing rate rather than the neuron type. These data were published in the *Journal of Physiology* (Kim and Dudek, 1993).

(ii) Non-chemical-synaptic mechanisms of synchronization

It was widely assumed that communication between neurons in the hypothalamus is mediated primarily by Ca^{2+} -dependent synaptic transmission. Simultaneous extracellular recordings of neuronal action potentials in the SCN demonstrated synchronized bursts of action potentials in Ca^{2+} -free medium, which blocks chemical synaptic transmission and increases membrane excitability. These periodic bursts of synchronized action potentials occurred across a large population of SCN neurons and persisted in the presence of antagonists of NMDA, non-NMDA and GABA_A receptors. Whole-cell recordings confirmed that postsynaptic potentials were blocked in this solution. These data provided strong evidence that mechanisms other than Ca^{2+} -dependent synaptic transmission can synchronize neurons in the mammalian SCN. A paper summarizing these results was published in the *Proceedings of the National Academy of Sciences U.S.A.* (Bouskila and Dudek, 1993).

(iii) Modeling of circadian rhythms of electrical activity

The firing rate of a population of SCN neurons *in vivo* shows stable circadian oscillations, but the length of the period of the circadian rhythm of firing of individual neurons is not known and may be different or similar to the population rhythm. We undertook a mathematical modeling study to assess this issue. We used published data from Bos and Mirmiran (1990) that reported different period lengths and amplitudes of rhythm oscillations for individual neurons recorded in

explant cultures of the SCN. We reconstructed the individual rhythms and extrapolated them for several cycles. We then calculated the combined rhythm of a population of these cells, and tested its stability. The period and amplitude of the rhythm of groups of neurons with different period lengths were unstable. Furthermore, the stability of the rhythm was reduced as the number of neurons increased. These results suggest that the stable circadian rhythm reported for neuron populations in the intact SCN emerges from the identical period length of individual neurons. Intercellular interactions in the SCN may underlie the stable circadian rhythm. A brief report on this work was published in *Brain Research* (Bouskila and Dudek, 1995a).

(iv) Patch-clamp analysis of K⁺ currents in SCN neurons

K⁺ currents are a primary, intrinsic mechanism for controlling firing rate, and are likely candidates for regulating the circadian rhythm of electrical activity. We sought to answer two general questions about K⁺ currents in SCN neurons: Which types of K⁺ currents are present, and are they consistently found in neurons throughout the SCN? Previous studies with sharp intracellular electrodes, including work from our laboratory (Kim and Dudek, 1993), had suggested the presence of a Ca²⁺-activated K⁺ current and an inward rectifier K⁺ current in some SCN neurons. We blocked voltage-dependent Na⁺ and Ca²⁺ currents with pharmacological treatments that allowed us to isolate Ca²⁺-independent K⁺ currents using whole-cell patch-clamp recording. A novel delayed outward-rectifier K⁺ current showed extremely fast activation (i.e., one to two orders of magnitude faster than similar currents in other mammalian central neurons) and slow inactivation. A transient outward K⁺ current (A-current) was also found. The A-current in SCN was very similar to A-currents in other areas of the brain. These two types of currents appeared to be present in all neurons throughout the SCN. This was determined by mapping the position of all recorded neurons. These K⁺ currents are likely to play a critical role controlling the shape of the action potential (delayed rectifier) and firing frequency (A-current); both currents are candidates for the underlying mechanism responsible for circadian changes in firing rate. These data were published in the *Journal of Physiology* (Bouskila and Dudek, 1995b).

(v) Local synaptic circuits in the SCN

We used three experimental approaches to test the hypothesis that SCN neurons form a system of local GABA-ergic circuits. First, the addition of TTX to block action-potential-dependent synaptic activity decreased the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) in all cells ($n = 7$, χ^2 test), and decreased the amplitude in 2 of 7 cells (Kolmogorov-Smirnov analysis of cumulative frequency distributions). Second, we selectively stimulated SCN neurons using focal pressure microapplication of glutamate, and observed that stimulation produced synaptic currents in other SCN neurons (12 of 32 neurons responded to such microstimulation with an increase in IPSC frequency).

A third approach in exploring local circuits was designed to examine additionally whether the probability of finding synaptic connections among cells was dependent on the sites of stimulation and recording within the SCN. Even

after further refinement of the pressure microapplication system, we determined that this method could not provide optimal spatial resolution. Although we are fairly certain that most of the stimulatory effects of glutamate pressure application were limited to the SCN, we could not be certain of the precise limits of our stimulation within the nucleus. To provide a more detailed and rigorous analysis of the local circuits in the SCN, we also began using the technique of photolysis of caged glutamate (Callaway and Katz, 1993) in which UV light (xenon flashlamp source) can be focused to a relatively small ($\sim 75 \mu\text{m}$) spot in the center of the translucent tissue slice ($\sim 400 \mu\text{m}$ -thick) containing the SCN. Slices were bathed in a solution containing caged glutamate, which was activated (uncaged) by a brief flash of UV light. Whole-cell voltage-clamp recording (using the blind patch technique) was used to detect increases in IPSC frequency. This more precise method of focal stimulation of local circuits with caged glutamate also demonstrated synaptic connectivity via GABA-mediated synapses in the SCN. This work is in press in the *Journal of Neurophysiology* (Strecker, Wuarin and Dudek, 1997).

(vi) Modulation of GABA_A-mediated currents in SCN neurons

We have probed the functional characteristics of GABA_A-receptor-mediated IPSCs in the SCN using whole-cell voltage-clamp techniques. IPSCs were recorded while Zn²⁺ was introduced into the bathing medium. We found that Zn²⁺ (200 μM) reduced the amplitude of IPSCs in most SCN neurons. Lower concentrations of Zn²⁺ (to 50 μm) have also been tested and found effective. We have verified the ability of Zn²⁺ to block GABA currents in experiments where GABA was microapplied. Zn²⁺ reduced responses to endogenous (synaptically released) and exogenous (microapplication) GABA, suggesting a postsynaptic mechanism. Surprisingly, on two cells (2 of 14) Zn²⁺ had a completely different effect, dramatically increasing both the frequency and amplitude of IPSCs. This effect, however, is rare and is difficult to reproduce. These data were presented last year at the annual meeting of the Society for Neuroscience (Strecker and Dudek, 1996).

Structure-function studies from various laboratories (e.g., Draguhn et al., 1990) indicate that high sensitivity to Zn²⁺ is often characteristic of GABA_A receptors lacking a γ subunit. Furthermore, the absence of this subunit generally precludes receptor sensitivity to benzodiazepines, such as valium (diazepam) or flunitrazepam. Benzodiazepines, when effective, potentiate GABA_A receptor-mediated currents. We found that IPSC amplitude or frequency in most neurons is unaffected by flunitrazepam (10 μM). However, IPSC delay constants were generally increased as were the amplitudes of GABA currents evoked by GABA microapplication. Taken together, these results suggest that SCN GABA_A receptors contain γ subunits, in contrast to reports from cultured SCN cells which suggested that these receptors were of the γ -less type. Further experiments with different concentrations of zinc and benzodiazepines are ongoing and should help to clarify this issue. These data will be presented at the upcoming annual meeting of the Society for Neuroscience (Strecker, Park and Dudek, 1997b in press)

(vii) Patch-clamp recording in thick SCN slices with attached optic nerves

We have conducted preliminary experiments to study the modulation of optic nerve input to the SCN. In order to record from SCN neurons and stimulate their synaptic input (i.e., the optic nerve), the SCN and the nerve must be cut such that both remain within the plane of section of the tissue slice. This requires thicker slices (~ 400 μm), which make it difficult to see individual neurons using our thin-slice imaging system. Thus, the blind-patch technique is being used in these experiments instead to probe serotonergic modulation of retinal input to the SCN.

B. Other hypothalamic regions

Under partial support from the AFOSR, we also undertook electrophysiological studies of other hypothalamic nuclei. This series of studies on other regions of the hypothalamus were supported primarily from other sources, and were largely conducted during our previous grant; however, we are including them here because they provide an important framework of our studies on the SCN and much of the manuscript preparation was completed during this grant.

(i) Paraventricular nucleus (PVN)

The purpose of these experiments was to test the hypothesis that neurons in the paraventricular nucleus (PVN) receive GABA-mediated inhibitory synaptic input from neurons in the general region surrounding the PVN. Intracellular recordings were obtained from neurons in and around the PVN, and glutamate microdrops were applied to slices dorsal, lateral, and ventral to the PVN to selectively activate local presynaptic neurons. Local glutamate microapplication increased the frequency and amplitude of IPSPs, and these IPSPs were blocked by bath application of the GABA_A receptor antagonist bicuculline. These data provide evidence that local synaptic circuits are primarily inhibitory and supplied by perinuclear GABA-ergic neurons, and that both magnocellular and parvicellular subpopulations in the PVN receive these local inhibitory synaptic inputs. These data suggest that local inhibitory neurons provide feedforward inhibition for excitatory inputs from other brain regions. This research was published in the *Journal of Physiology* (Tasker and Dudek, 1993).

(ii) Supraoptic nucleus (SON)

Patch-clamp recording techniques allowed us to test more rigorously the hypothesis that GABA and glutamate mediate excitatory and inhibitory postsynaptic currents in the supraoptic nucleus (SON). A quantitative analysis of EPSCs and IPSCs showed that these events were blocked by the non-NMDA receptor antagonist CNQX, and the IPSCs were blocked by the GABA_A receptor antagonist bicuculline. These results suggest that in the *in vitro* slice preparation, glutamate mediates all the spontaneous EPSCs in magnocellular neurosecretory cells at resting potential by acting primarily on AMPA/kainate type receptors (i.e., non-NMDA receptors), and that activation of GABA_A receptors mediates most if not all IPSCs. The results of this research were published in the *Journal of*

Neuroscience (Wuarin and Dudek, 1993).

(iii) Preoptic area

A series of experiments were performed with both sharp intracellular and whole-cell patch-clamp recordings in the preoptic area of the hypothalamus. The initial sharp intracellular recordings were correlated with intracellular staining data using biocytin. Even though the intracellular staining showed that recordings were obtained from neurons of diverse morphology, nearly all the neurons had similar electrophysiological properties. In particular, low-threshold Ca^{2+} spikes and linear or nearly linear current-voltage relations were obtained in virtually all neurons. Most neurons had fast EPSPs and IPSPs, and the fast IPSPs were blocked by bicuculline and had a reversal potential near the Cl_- equilibrium potential. This research was published in the *Journal of Comparative Neurology* (Hoffman, Kim, Gorski, and Dudek, 1994a).

Subsequent studies using whole-cell patch-clamp recordings showed that spontaneous EPSPs and IPSCs were blocked by bath application of CNQX and bicuculline, providing further evidence that glutamate and GABA mediate virtually all of the fast synaptic currents in the medial preoptic area. This latter paper was published in *Brain Research* (Hoffman, Wuarin and Dudek, 1994b).

REFERENCES (SEE ALSO PUBLICATIONS)

Bos, N.P.A. and Mirmiran, M. (1990) Circadian rhythms in spontaneous neuronal discharge of the cultured suprachiasmatic nucleus. *Brain Res.*, 511:158-162.

Callaway, E.M., and Katz, L.D. (1993). Photostimulation using caged glutamate reveals functional circuitry in living brain slices. *Proc. Natl. Acad. Sci. USA*, 90:3207-3210

Draguhn, A., Verdoorn, T.A., Ewert, M., Seeburg, P.H., and Sakmann, B. (1990). Functional and molecular distinction between recombinant rat GABA_A receptor subtypes by Zn^{2+} . *Neuron*, 5:781-788.

4. PROFESSIONAL PERSONNEL

- Mr. Yona Bouskila (graduate student)
- Dr. F. Edward Dudek (principal investigator)
- Dr. Neil H. Hoffman (postdoctoral fellow)
- Dr. G. Joseph Strecker (postdoctoral fellow)
- Dr. Jeffery Tasker (postdoctoral fellow)
- Dr. Jean-Pierre Wuarin (collaborative investigator)

5. PUBLICATIONS

Peer-reviewed:

Suprachiasmatic Nucleus

Kim, Y.I. and Dudek, F.E. (1993) Membrane properties of rat suprachiasmatic nucleus neurons receiving optic nerve input. J. Physiol., London 464:229-243.

Bouskila, Y. and Dudek, F.E. (1993) Neuronal synchronization without active calcium-dependent synaptic transmission in the hypothalamus. Proc. Nat. Acad. Sci. USA 90:3207-3210.

Dudek, F.E., Kim, Y.I. and Bouskila, Y. (1993) Electrophysiology of the suprachiasmatic nucleus: synaptic transmission, membrane properties, and neuronal synchronization. J. Biol. Rhythms 8(Supplement):S33-S37 (invited, refereed review).

van den Pol, A.N. and Dudek, F.E. (1993) Cellular communication in the biological clock, the suprachiasmatic nucleus. Neuroscience 56:793-811.

Bouskila, Y. and Dudek, F.E. (1995a) Can a population of suprachiasmatic nucleus neurons with different period lengths produce a stable circadian rhythm? Brain Res. 670:333-336.

Bouskila, Y. and Dudek, F.E. (1995b) A rapidly activating type of outward rectifier K⁺ current and A-current in rat suprachiasmatic nucleus neurones. J. Physiol., London 488:339-350.

Strecker, G.J., Wuarin, J.-P. and Dudek, F.E. (1997) GABA_A-mediated local synaptic pathways in the rat suprachiasmatic nucleus. J. Neurophysiol., in press.

Other Hypothalamic Areas

Wuarin, J.P. and Dudek, F.E. (1993) Patch clamp analysis of spontaneous synaptic currents in supraoptic neuroendocrine cells of the rat hypothalamus. J. Neurosci. 13:2323-2331.

Tasker, J.G. and Dudek, F.E. (1993) Local inhibitory synaptic inputs to neurones of the paraventricular nucleus in slices of rat hypothalamus. J. Physiol., London 469:179-192.

Hoffman, N.W., Kim, Y.I., Gorski, R.A. and Dudek, F.E. (1994a) Homogeneity of electrophysiological properties in different neuronal subtypes in medial preoptic slices containing the sexually dimorphic nucleus of the rat. J. Comp. Neurol. 345:396-408.

Hoffman, N.W., Wuarin, J.P. and Dudek, F.E. (1994b) Whole-cell patch-clamp recordings of spontaneous synaptic currents in medial preoptic neurons in rat

hypothalamic slices: mediation by amino acid transmitters. Brain Res. 660:349-352.

Chapters:

Strecker, J.G., Bouskila, Y. and Dudek, F.E. (1995) Neurotransmission and electrophysiological mechanisms in the suprachiasmatic nucleus. Seminars in the Neurosciences, Ed. By K. Siwicki, 7:43-51.

van den Pol, A.N., Strecker, G.J. and Dudek, F.E. (1996) Excitatory and inhibitory amino acids and synaptic transmission in the suprachiasmatic nucleus. Prog. Brain Res. 111:41-56.

Bouskila, Y., Strecker, J., and Dudek, F.E.. Cellular mechanisms of circadian function in the SCN. Handbook of Behavioral Neurobiology -- Circadian Clocks, Ed. by J.S. Takahashi, F.W. Turek and R.Y. Moore (in press).

Abstracts:

Bouskila, Y. and Dudek, F.E. (1993) Non-chemical synaptic mechanisms synchronize neuronal activity in the suprachiasmatic nucleus (SCN). Sleep Research 22:613.

Bouskila, Y. and Dudek, F.E. (1993) Whole-cell patch-clamp analysis of neuronal excitability in the suprachiasmatic nucleus (SCN). Soc. Neurosci. Abstr. 19:1613, #662.1.

Strecker, G.J. and Dudek, F.E. (1994) Local synaptic circuits in the rat suprachiasmatic nucleus. Soc. Neurosci. Abstr., 20:1439

Strecker, G.J., and Dudek, F.E. (1996) Zinc-sensitivity of GABA_A-mediated synaptic currents in the rat suprachiasmatic nucleus. Soc. Neurosci. Abstr., 22, 1139.

Strecker, G.J., Park, W.K. and Dudek, F.E. (1997) Flunitrazepam potentiates GABA_A receptor-mediated currents in the rat suprachiasmatic nucleus. Soc. Neurosci. Abstr., in press.

6. INTERACTIONS/TRANSITIONS

Participation/presentations at meetings, conferences, etc.

March 13-17, 1993 -- International Conference on the Cellular Consequences of Sleep in Maui, Hawaii. Title - "Electrophysiology of the Suprachiasmatic Nucleus: Synaptic Transmission, Membrane Properties, and Neuronal Synchronization" organized by Dr. J. Miller.

June 5, 1993 -- Workshop on the "Suprachiasmatic Nucleus as an in vitro Model System" at the University of Virginia in Charlottesville, Virginia. Organized by G. D. Block.

April 30-May 5, 1995 -- Gordon Conference on Chronobiology in Barga, Italy.
Title--"Electrophysiological properties of suprachiasmatic nucleus neurons in hypothalamic slices." Organized by S. Daan and G. Block.

August 28-September 9, 1995--International Summer School of Brain Research on Hypothalamic Integration of Circadian Rhythms. Title--"SCN input and internal synchronization." Organized by R.M. Buijs.

January 29, 1996--Winter Conference on Brain Research Workshop on Role of GABA in Suprachiasmatic Nucleus and Circadian Rhythm Generation in Snowmass, Colorado. Organizer.

May 9, 1996--Society for Research on Biological Rhythms Symposium on Building a Suprachiasmatic Nucleus from Multiple Oscillators in Jacksonville, Florida. Title--"Local neuronal interactions in the suprachiasmatic nucleus." Organized by K. Honma.

In collaboration with Cell Robotics Inc., we are developing a laser-based method for photolysis of caged glutamate in our laboratory. We have received a nitrogen pulsed-laser and an argon laser, and are now testing them. We have become a development and test site for this application.

7. NEW DISCOVERIES, INVENTIONS OR PATENT DISCLOSURES

None

8. HONORS/AWARDS

Javits Neuroscience Investigator Award 1987-1994